Analysis of Total PCBs and PCB Congeners and Trans-nonachlor in Fish by Gas Chromatography/ Negative Chemical Ionization Single Ion Mass Spectrometry

Standard Operating Procedure SOP No. HC 519.D
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1.0 Scope and Application

1.1 This method covers the determination of total PCBs, PCB congeners and Trans-nonachlor developed at the National Biological Service/Great Lakes Science Center (NBS/GLSC) by gas chromatography using negative chemical ionization single ion monitoring mass spectrometry (GC/NCI/SIM) for the U.S. EPA Mass Balance Study. The parameters presently reported by this method are given in Table 1. The parameters listed are qualitatively and quantitatively determined as target compounds by this method. The NCI reagent gas used is methane.

1.2 The method detection limits (MDL) for selected congeners are also listed in Table 1. These were determined according to EPA rule Appendix B of 40 CFR Part 136 on method blanks spiked with one congener at each chlorine level (one through ten) at very low levels (3-10X signal to noise ratio) on the GC/MS just before extraction. The value for the other congeners at a given level of chlorination were calculated using the ratio of the normal response factor for the congener used in the MDL study for that level of chlorination to the normal response factor of the congener in question, multiplied by the detection limit of that congener representing the chlorine level.

2.0 Summary of Method

This method covers only the analytical portion of the testing procedure applicable to fish. Sampling and sample preparation procedures for PCBs and trans-nonachlor in these matrices are already in place and are covered by the appropriate NBS/GLSC methods. Qualitative identification the parameters in the resulting extracts is performed using the retention time and the relative abundance of two characteristic masses (m/z). Quantitative analysis is performed using an internal standard technique with a single characteristic m/z.

3.0 Interferences

3.1 Interferences from sample preparation glassware and reagents are routinely monitored by running method blanks. The method blank is run through the entire extraction process along with the samples, except that it consists only of sodium sulfate, the compound that is mixed with fish tissue before extraction.

3.2 Matrix interferences may be caused by compounds that are co-extracted from the sample, and may vary considerably from source to source. The level of interference using GC/NCI/SIM is far less, however, than when using standard positive electron-impact (EI) mass spectrometry.

3.3 Two congeners of interest, numbers 77 and 126, are not separated completely from interfering PCBs. For the Mass Balance Study, they will be quantitated by subtracting out the typical contribution of the interfering PCB and will thus be quantitated somewhat less accurately than the other target congeners. Neither compound contributes significantly to total PCBs in biota.

4.0 Safety

4.1 PCBs have been tentatively classified as known or suspected, human or mammalian carcinogens. Primary standards of these toxic compounds must be handled in a manner to avoid direct contact.

4.2 The toxicity or carcinogenicity of each chemical and reagent used in this method has not been precisely defined, although each chemical compound should be treated as a potential health hazard. The NBS/GLSC maintains a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets is also available to all personnel involved in chemical analysis.

5.0 Apparatus

5.1 Gas Chromatograph - The NBS/GLSC uses an HP5890 gas chromatograph (GC) equipped with an HP7673A robotic autosampler. All data are acquired using computer controlled batching of sample, standard, and quality control runs. This approach is critical to obtaining the retention time reproducibility needed for doing PCB congener work, even though relative retention times are used. Members of a given congener elute closely enough together that tight control of chromatographic conditions is necessary to avoid misidentification, as the ion ratios for a given congener series are very similar. The HP gas chromatograph is capable of multi-stage temperature programming (ramping) and is equipped for splitless/split capillary injection.

5.2 Column - A 30 meter DB-5 fused silica capillary column is used, with an I.D. of .25 mm and a coating thickness of .25 micron.

5.3 Mass Spectrometer - The NBS/GLSC uses an HP5988A research-grade low resolution mass spectrometer (MS) equipped with positive and negative chemical ionization capability. The instrument can perform single ion monitoring (SIM), analyzing up to 999 ion groups of 20 ions per group during each run. The GC capillary column is interfaced directly into the MS source with no splitting of carrier gas.

5.4 Data System - The HP5988A mass spectrometer is equipped with an RTE-A data system, capable of doing automatic identification and quantification of target compounds using a reverse search for identification and an internal standard method for quantitation. This system also controls data acquisition, including automatic operation of the GC, MS, and autosampler, and any other required manipulation of the raw data or processed files. The HP Enviroquant software is used for data reduction, which will necessitate transfer of raw data files from RTE to PC/DOS by RS-232 using a procedure file.

6.0 Analytical Procedure

6.1 Tuning and Calibration--The source is operated in the CI mode using methane reagent gas. When the source pressure has stabilized at a value known to produce satisfactory results (.5 to .8 Torr), the instrument is manually tuned and calibrated using an NCI customized tuning file. Pressure stabilization usually takes about 45 minutes. Tuning is checked daily by using at least two ions which span the approximate range of interest for PCBs/trans-nonachlor. Using FC-43, the ions currently selected are 235 and 452. A third ion, 633, is always present in the display, but is not used for tuning. The parameter ramp program is run for purposes of maximizing NCI response, with the HP lenses known as repeller, drawout, ion focus, and entrance lens being adjusted so that their settings are near the displayed curve maxima for the two ions. Since the lens settings are interactive, the lens sequence should be rechecked until no changes to the settings are needed. The manufacturer's manual contains detailed instructions on manual tuning in NCI. Once tuning is accomplished, a half-page profile scan is printed out. The source pressure and any unusual conditions is noted on this display, and the exact masses of ion 452 is checked to assure that it has not changed more than 0.15 amu from 452.0. Any centroid change greater than this may require alteration of the exact masses as given in the ion groupings (presented in Table 3) of this method. It is much simpler to adjust the centroid in the tuning file to within 0.15 of 452.0 with the Mass Offset (B/b) control in the manual tuning procedure. Once proper tuning and calibration have been obtained, the manual tune is saved before exiting the manual tuning program. Note that tuning is most needed the first few weeks after the source has been cleaned. The system becomes very stable thereafter, and usually no tuning operations are necessary other than printing out and archiving a copy of the tuning display.

6.2 Calibration and Linear Range

6.2.1 GC and MS conditions are set by the analytical method. These conditions are given in Table 2 for the GC parameters and in Table 3 for the MS parameters. It is important to note that the method file controls if there is a disparity between it and the tuning file with respect to source temperature, multiplier voltage, and/or emission current. The column head pressure is manually controlled using the GC oven pressure controller and the oven may be baked by setting the oven temperature from the keyboard if no method is running. Source pressure (reagent gas flow) is controlled manually using the CI reactant gas flow controller. All other parameters are established through the terminal and keyboard.

6.2.2 Standards of a 25:18:18 ratio of Aroclor 1232, 1248, and 1262 are run at concentrations of 500, 2500, and 5000 ng/g to demonstrate linear response over this range. Using these concentrations, we obtain three concentrations for all congeners which do not saturate at the highest concentration and do not go below the detection limit for the least concentrated congener. A linear three-point curve is established when the standard deviation of the relative response ratios at the three concentrations is less than 25%.

6.2.3 The 2500 PPB concentration is designated as the calibration standard. The concentrations in the ID file are based on this standard. It is run at the beginning of each batch run. The performance standard is be run immediately following the calibration standard. Congener concentrations in the performance standard are calculated from the calibration standard response factors. A subset of six congeners are used to evaluate the current response factors. These include #44, #207 (small peaks), #101, #185 (average peaks, and #151, #180 (large peaks). Calculated concentrations of these congeners are compared to their known concentrations. Deviations from actual concentrations of greater than 50% for the small peaks and greater than 10% for the average and large peaks result in flagging of all samples in the data set for the failed congener.

6.2.4 Because a single calibration standard is being used to generate RRFs, the calibration standard concentration should be within a factor of five of the concentrations of PCBs in the sample extracts. Sample extracts that fall outside this range are either diluted or concentrated to bring them to within this range.

6.3 Sample Run

Once the source pressure has stabilized at the desired value and a manual tune has been performed (or checked), a batch data acquisition (sample run) can be initiated. A batch data acquisition is begun by accessing the batch sequence menu. Every batch includes a method blank, standards, a spike standard, a surrogate standard, a check sample, a background fish extract, then actual samples and spikes. The latter will vary with the number of samples extracted at one time. Information on each sample is entered sequentially into the menus as they come up until the batch edit. The proper tuning file, method file, allowable space per run, and total cartridge space (minimum 600 tracks for a normal batch) for the batch are also inputted to the batch file. When finished, the batch sequence listed and checked for correctness, the batch is started. Before the start command is given, a final check is made as to condition of column, solvent wash vials, septum, injection port liner, and leak-free status of the injection area. Internal standards should be spiked into the samples and spikes just before the beginning of the batch data acquisition run.

6.4 Data Reduction and Reporting

6.4.1 Quantification of PCBs is congener specific and done by the internal standard method. The internal standards that will be used are congeners #136 and #204. Congeners eluting prior to and including #110 are quantitated relative to internal standard #136, and those after #110 are quantitated relative to #204.

6.4.2 Data analysis begins with the execution of AUTSFX (Single ion monitoring background subtraction program) for the entire batch and then BATCH3 using ID file MBSUR for the entire batch. This will provide assurance that the batch completed without gross problems such as syringe lugging or failure of GC temperature controlled zones. The CENTROIDS procedure file is the first injection (DOSE) to verify calibration of mass axis. A procedure file is then run on the standard PCB solution calibration standard to check source condition and GC resolution. The appropriate data are recorded in a log book. The batch program is then executed to produce the QT output file needed to perform a calibration check. If many compounds are absent, it usually means the internal standard retention times must be adjusted in the ID file. Ion ratios almost never have to be adjusted. Even if all compounds are present after adjusting the ID file, the retention times should be closely checked to assure that they are no more than 0.2 minute from expected. Once the QT output file is acceptable for all standards runs, the calibration is checked.

6.4.3 At this time, spike and surrogate recoveries are calculated and examined for acceptability insofar as method performance is concerned. Surrogates (PCBs #65 and #166) are spiked into every sample and blank. The matrix spike is spiked into a hatchery fish at 30 times background PCB levels. These spikes are made directly into the fish/sodium sulfate mixture immediately before solvent extraction. For a given sample set, acceptance values for spike recoveries are specified in Table 7.1 of the Mass Balance QAPjP. If matrix spike recoveries do not meet these standards, then data from that sample set are flagged. If surrogate spike recoveries do not meet these standards, then that sample must be evaluated according to the QAPjP. If the flags are determined to be serious samples must be re-extracted and analyzed. The spike ID file is MBSPK. The surrogate ID file is MBSUR.

6.4.4 Once checking of standards runs is satisfactorily completed, the ID file is updated for a final time using the response factors from the calibration standard by executing the QCAL command file. The final QT reports are then produced. The value for the internal standards in the ID file may have to be adjusted to reflect differing final volumes or initial weights.

6.4.5 At this point a comparison of duplicates is made, and results for the check fish run during the batch are compared to values representing an average of six or more check fish ran previously. The acceptance values for duplicates and for the check fish are specified in Table 7.1 of the NBS/CLSO QAPjP.

7.0 Method Performance

As part of NBS-GLSC internal ongoing quality control and performance monitoring, check fish samples are run as deemed to be necessary (usually one per sample batch). These are run as ordinary samples and the results are tracked for consistency over time. Baseline data for check fish samples are generated by a one time extraction of six replicate lab reference fish samples. The replicates are processed through sample preparation and analyzed in the normal manner. The concentration and standard deviations (N=6) are calculated for the parameters routinely analyzed. Variation in the check fish samples over the course of many extraction batches can be expected to be no better, at best, than this baseline data.

Table 1. PCB Congeners/trans-nonachlor to be Determined by GC/NCI/SIM.

	Compound	Instrument Detection Limit using 1 g sample (ng/g)
1.	PCB Congener #31+#28	9
2.	PCB Congener #33	4
3.	PCB Congener #22	4
4.	PCB Congener #52	12
5.	PCB Congener #49	18
6.	PCB Congener #47+#48	6
7.	PCB Congener #44	25
8.	PCB Congener #42	4
9.	PCB Congener #41+#71	18
10.	PCB Congener #64	4
11.	PCB Congener #40	7
12.	PCB Congener #63	0.4
13.	PCB Congener #74	2
14	PCB Congener#70 + #76	1
15.	PCB Congener #66	2
16.	PCB Congener #95	6
17.	PCB Congener #91	7
18.	PCB Congener #56+#60	1
19.	PCB Congener #84+#92+#89	1
20.	PCB Congener #101	0.2
21.	PCB Congener #99	0.4
22.	Trans-nonachlor	0.08
23.	PCB Congener #119	0.1
24.	PCB Congener #83	0.6
25.	PCB Congener #97	0.9
26.	PCB Congener #81 + #87	0.6
27.	PCB Congener #85	0.3
28.	PCB Congener #77	0.2
29.	PCB Congener #110	0.5
30.	PCB Congener #82	1
31.	PCB Congener #151	0.02
32.	PCB Congener #144 + #135	0.03
33.	PCB Congener #107	0.3
34.	PCB Congener #123	0.1
35.	PCB Congener #149	0.04
36.	PCB Congener #118	0.3
37.	PCB Congener #134	0.02
38.	PCB Congener #114	0.4
39.	PCB Congener #131	0.01
40.	PCB Congener #146	0.01
41.	PCB Congener #132 + #153	0.02
42.	PCB Congener #105	0.02
43.	PCB Congener #141	0.1

Table 1. PCB Congeners/trans-nonachlor to be Determined by GC/NCI/SIM. (Cont'd)

	Compound	Instrument Detection Limit using 1 g sample (ng/g)
44.	PCB Congener #137 + #176	0.08
45.	PCB Congener #138 + #163	0.04
46.	PCB Congener #158	0.03
47.	PCB Congener #129	0.01
48.	PCB Congener #126	0.03
49.	PCB Congener #178	0.1
50.	PCB Congener #175	0.1
51.	PCB Congener #187 + #182	0.08
52.	PCB Congener #183	0.06
53.	PCB Congener #128	0.02
54.	PCB Congener #167	0.03
55.	PCB Congener #185	0.04
56.	PCB Congener #174	0.09
57.	PCB Congener #177	0.1
58.	PCB Congener #202	0.2
59.	PCB Congener #171	0.1
60.	PCB Congener #156	0.04
61.	PCB Congener #173	0.06
62.	PCB Congener #157	0.03
63.	PCB Congener #200	0.2
64.	PCB Congener #172	0.04
65.	PCB Congener #197	0.04
66.	PCB Congener #180	0.07
67.	PCB Congener #193	0.08
68.	PCB Congener #191	0.1
69.	PCB Congener #199	0.2
70.	PCB Congener #170 + #190	0.09
71.	PCB Congener #198	0.1
72.	PCB Congener #201	0.3
73.	PCB Congener #203 + #196	0.4
74.	PCB Congener #189	0.1
75.	PCB Congener #195	0.1
76.	PCB Congener #208	0.07
77.	PCB Congener #207	0.1
78.	PCB Congener #194	0.1
79.	PCB Congener #205	0.2
80.	PCB Congener #206	0.2
81.	PCB Congener #209	0.07

Table 2. GC and Autosampler Operating Parameters

1. Column - 30 meter DB-5 (J&W Scientific), .25 mm I.D., .25 micron film thickness.

2. GC Temperature Program - Initial temperature 80°C, hold for one minute, then program to 150° at 20°/minute, then program to 250° at 2°/minute, hold five minutes. Post-run bakeout is 300° for six minutes.

3. Oven Equilibration Time - three minutes
   Total Run Time - 59 minutes
   Scanning Start Time - four minutes
   Splitless Operation Time - two minutes

4. Injection Port Temperature - 280°C
   GC/MS Interface Temperature - 280°C

5. Sample Injection Volume - 2 microliters. Data Cartridge Space - Minimum 600 tracks available space for a 24-run batch. Minimum Blocks Reserved for CR Check - 3000.

6. Carrier Gas - Helium at 10-15 psi column head pressure. This can vary, depending mostly on column age.

Table 3. Mass Spectrometer Operating Parameters

1. Source Temperature - 110°C

2. Multiplier Voltage - 1400-2800V (depending on stage of multiplier life)

3. Emission Current - 300 µA

4. Electron Energy - 200 eV

5. Reagent Gas - Methane

6. Source Pressure - (0.5 to 0.8 Torr).

7. SIM Groupings - Group 1, run from 4.0 to 16.3 minutes
   Group 2, run from 16.3 to 21.5 minutes
   Group 3, run from 21.5 to 26.0 minutes
   Group 4, run from 26.0 to 30.0 minutes
   Group 5, run from 30.0 to 35.5 minutes
   Group 6, run from 35.5 to 39.85 minutes
   Group 7, run from 39.85 to 45.6 minutes
   Group 8, run from 45.6 to 46.3 minutes
   Group 9, run from 46.3 to 50.0 minutes
   Group 10, run from 50.0 to 59.0 minutes

Table 3. Mass Spectrometer Operating Parameters (Cont'd)

Group 1	Exact masses 254.8, 254.9, 255, 256.8, 256.9, 257 (Lindane) each at dwell times of 150 milliseconds.
Group 2	Exact masses 255.86, 255.87, 255.88, 255.89, 256.9 256.91, 256.92, 256.93, 256.94, 256.95, 257.86, 257.87, 257.88, 257.89, 257.9, 257.91, 257.92, 257.93, 257.94, 257.95 (Trichlorobiphenyls) each at dwell times of 45 milliseconds.
Group 3	Exact masses 289.86, 289.87, 289.88, 289.89, 289.9 289.91, 289.92, 289.93, 289.94, 289.95, 291.86, 291.87, 291.88, 291.89, 291.9, 291.91, 291.92, 291.93, 291.94, 291.95 (Tetrachlorobiphenyls) each at dwell times of 45 milliseconds.
Group 4	Exact masses 289.79, 289.8, 289.9, 291.79, 291.8, 291.9 (Tetrachlorobiphenyls), Exact masses 325.79, 325.8, 325.9, 327.79, 327.8, 327.9 (Pentachlorobiphenyls), Exact masses 441.6, 441.7, 443.6, 443.7 (Trans-nonachlor) each at dwell times of 55 milliseconds.
Group 5	Exact masses 289.79, 289.8, 291.79, 291.8 (Tetrachlorobiphenyls), Exact masses 315.8, 315.9, 317.8, 317.9(4,4'-DDE), Exact masses 325.8, 325.9, 327.8, 327.9 (Pentachlorobiphenyls), Exact masses 359.7, 359.8, 358.9, 361.8 (Hexachlorobiphenyls), Exact masses 379.7, 379.8, 381.7, 381.8 (Dieldrin) each at dwell times of 45 milliseconds.
Group 6	Exact masses 325.77, 325.78, 325.79, 325.8, 325.81, 325.82, 327.77, 327.78, 327.79, 327.8, 327.81, 327.82, (Pentachlorobiphenyls), Exact masses 359.7, 359.8, 361.7, 361.8 (Hexachlorobiphenyls), Exact masses 393.6, 393.7, 395.6, 395.7 (Heptachlorobiphenyls) each at dwell times of 45 milliseconds.
Group 7	Exact masses 359.69, 359.7, 359.8, 361.69, 361.7, 361.8, (Hexachlorobiphenyls), Exact masses 393.59, 393.6, 393.7, 395.59, 395.6, 395.7 (Heptachlorobiphenyls), Exact masses 427.59, 427.6, 427.7, 429.59, 429.6, 429.7 (Octachlorobiphenyls) each at dwell times of 50 milliseconds.
Group 8	Exact masses 359.66-359.74 (10 ions) and exact masses 359.76-359.84 (10 ions), each at dwell times of 45 milliseconds (PCB #169).
Group 9	Same exact masses and dwell times as Group 7, except add exact masses 463.6, 465.6 (Nonachlorobiphenyls) and reduce each dwell time to 45 milliseconds.
Group 10	Exact masses 427.68, 427.69, 427.7, 427.71, 427.72, 429.68, 429.69, 429.7, 429.71, 429.72 (Octachlorobiphenyls), Exact masses 463.59, 463.6, 463.61, 465.59, 465.6, 465.61 (Nonachlorobiphenyls), Exact masses 497.49, 497.50, 499.49, 499.50 (Decachlorobiphenyl), each at dwell times of 55 milliseconds.